

What is claimed is:

1. A therapeutic composition comprising antigen presenting cells pulsed with a preparation comprising enucleated cytosol and cell membranes of cancer cells infected with a recombinant vaccinia virus encoding at least one immunostimulating molecule.
2. The composition of claim 1, wherein the enucleated cytosol is substantially free of nuclei.
3. The composition of claim 1, wherein the cell membranes comprise at least two HLA class I A antigens.
4. The composition of claim 1, wherein the recombinant vaccinia virus is either live or inactivated.
5. The composition of claim 1, wherein the immunostimulating molecule is IL-2.
6. The composition of claim 1, wherein the immunostimulating molecules comprises FLT-3 ligand, FLT-3/FLK-2 ligand, GM-CSF, G-CSF, IL-3, IL-4, IL-6, IL-7, IL-12, IL-15, IL-18, stem cell factor, an interferon, or a combination thereof.
7. The composition of claim 1 wherein the immunostimulating molecule comprises a melanoma immunogen selected from the group consisting of MAGE-1, MAGE-3, BAGE, GAGE, PRAME, NY-ESO-1, tyrosinase, Melan-A, MART- 1, gp 100, TRP- 1, TRP-2, MUM-1, CDK4, beta-catenin, gp 100in4, p15, N-acetylglucosaminyltransferase, B7-1, TA-90, lysosome-associated membrane protein, melanocyte-stimulating hormone receptor, p90 calnexin, and a combination thereof.
8. The composition of claim 1, wherein the antigen presenting cells are dendritic cells or monocytes.
9. The composition of claim 1, wherein the antigen presenting cells are dendritic cells and monocytes.
10. The composition of claim 1, wherein the antigen presenting cells are autologous cells.
11. The composition of claim 1, wherein the antigen presenting cells are HLA-matched to a host to be treated.
12. The composition of claim 1, wherein the cancer cells are melanoma cells.
13. The composition of claim 12, wherein the melanoma cells comprise one or more cells from the group consisting of Mel-2, Mel-3, Mel-4, Mel-6, and Mel-9.
14. The composition of claim 1, wherein the cancer cells are established cancer cell lines.
15. The composition of claim 1, wherein the cancer cells are selected from the group consisting

of fibrosarcoma, myxosarcoma, liposarcoma, chondrosarcoma, osteogenic sarcoma, chordoma, angiosarcoma, Kaposi's sarcoma, endotheliosarcoma, lymphangiosarcoma, lymphangioendotheliosarcoma, synovioma, mesothelioma, Ewing's tumor, leiomyosarcoma, rhabdomyosarcoma, rhabdosarcoma, colorectal carcinoma, pancreatic cancer, breast cancer, ovarian cancer, prostate cancer, squamous cell carcinoma, basal cell carcinoma, adenocarcinoma, sweat gland carcinoma, sebaceous gland carcinoma, papillary carcinoma, papillary adenocarcinomas, cystadenocarcinoma, medullary carcinoma, bronchogenic carcinoma, renal cell carcinoma, hepatoma, bile duct carcinoma, choriocarcinoma, seminoma, embryonal carcinoma, Wilms' tumor, cervical cancer, testicular tumor, lung carcinoma, small cell lung carcinoma, bladder carcinoma, epithelial carcinoma, glioma, astrocytoma, medulloblastoma, craniopharyngioma, ependymoma, pinealoma, hemangioblastoma, acoustic neuroma, oligodendroglioma, meningioma, neuroblastoma, retinoblastoma, myeloma, lymphoma, and leukemia cells.

16. The composition of claim 14, wherein the cancer cell lines are selected from the group of cancer cell lines consisting of fibrosarcoma, myxosarcoma, liposarcoma, chondrosarcoma, osteogenic sarcoma, chordoma, angiosarcoma, Kaposi's sarcoma, endotheliosarcoma, lymphangiosarcoma, lymphangioendotheliosarcoma, synovioma, mesothelioma, Ewing's tumor, leiomyosarcoma, rhabdomyosarcoma, rhabdosarcoma, colorectal carcinoma, pancreatic cancer, breast cancer, ovarian cancer, prostate cancer, squamous cell carcinoma, basal cell carcinoma, adenocarcinoma, sweat gland carcinoma, sebaceous gland carcinoma, papillary carcinoma, papillary adenocarcinomas, cystadenocarcinoma, medullary carcinoma, bronchogenic carcinoma, renal cell carcinoma, hepatoma, bile duct carcinoma, choriocarcinoma, seminoma, embryonal carcinoma, Wilms' tumor, cervical cancer, testicular tumor, lung carcinoma, small cell lung carcinoma, bladder carcinoma, epithelial carcinoma, glioma, astrocytoma, medulloblastoma, craniopharyngioma, ependymoma, pinealoma, hemangioblastoma, acoustic neuroma, oligodendroglioma, meningioma, neuroblastoma, retinoblastoma, myeloma, lymphoma, and leukemia cell lines.
17. The composition of claim 1, wherein the cancer cells are harvested from a host to be treated with the composition.

18.

An immunotherapeutic vaccine comprising:

- (a) a first part comprising a first recombinant vaccinia virus encoding at least one first

immunostimulating molecule; and

- (b) a second part comprising antigen presenting cells pulsed with a preparation comprising enucleated cytosol and cell membranes of cancer cells infected with a recombinant vaccinia virus encoding at least one second immunostimulating molecule.

19. The vaccine of claim 18, wherein the enucleated cytosol is substantially free of nuclei.
20. The vaccine of claim 18, wherein the cell membranes comprise at least two HLA class I A antigens.
21. The vaccine of claim 18, wherein the first recombinant vaccinia virus is a live virus.
22. The vaccine of claim 18, wherein the second recombinant vaccinia virus is either live or inactivated.
23. The vaccine of claim 18, wherein the first immunostimulating molecule is IL-2.
24. The vaccine of claim 18, wherein the first immunostimulating molecule comprises FLT-3 ligand, FLT-3/FLK-2 ligand, GM-CSF, G-CSF, IL-3, IL-4, IL-6, IL-7, IL-12, IL-15, IL-18, stem cell factor, an interferon, or a combination thereof.
25. The vaccine of claim 18, wherein the first immunostimulating molecule comprises a melanoma immunogen selected from the group consisting of MAGE-1, MAGE-3, BAGE, GAGE, PRAME, NY-ESO-1, tyrosinase, Melan-A, MART- 1, gp 100, TRP- 1, TRP-2, MUM- 1, CDK4, beta-catenin, gp 100in4, p15, N-acetylglucosaminyltransferase, B7-1, TA-90, lysosome-associated membrane protein, melanocyte-stimulating hormone receptor, p90 calnexin, and a combination thereof.
26. The vaccine of claim 18, wherein the second immunostimulating molecule is IL-2.
27. The vaccine of claim 18, wherein the second immunostimulating molecule comprises FLT-3 ligand, FLT-3/FLK-2 ligand, GM-CSF, G-CSF, IL-3, IL-4, IL-6, IL-7, IL-12, IL-15, IL-18, stem cell factor, an interferon, or a combination thereof.
28. The vaccine of claim 18, wherein the second immunostimulating molecule comprises a melanoma immunogen selected from the group consisting of MAGE-1, MAGE-3, BAGE, GAGE, PRAME, NY-ESO-1, tyrosinase, Melan-A, MART- 1, gp 100, TRP- 1, TRP-2, MUM- 1, CDK4, beta-catenin, gp 100in4, p15, N-acetylglucosaminyltransferase, B7-1, TA-90, lysosome-associated membrane protein, melanocyte-stimulating hormone receptor, p90 calnexin, and a combination thereof.

29. The vaccine of claim 18, wherein the antigen presenting cells are dendritic cells or monocytes.
30. The vaccine of claim 18, wherein the antigen presenting cells are dendritic cells and monocytes.
31. The vaccine of claim 18, wherein the antigen presenting cells are autologous cells.
32. The vaccine of claim 18, wherein the antigen presenting cells are HLA-matched to a host to be treated.
33. The vaccine of claim 18, wherein the cancer cells are melanoma cells.
34. The vaccine of claim 33, wherein the melanoma cells comprise one or more cells from the group consisting of Mel-2, Mel-3, Mel-4, Mel-6, and Mel-9.
35. The vaccine of claim 18, wherein the cancer cells are established cancer cell lines.
36. The vaccine of claim 18, wherein the cancer cells are selected from the group consisting of fibrosarcoma, myxosarcoma, liposarcoma, chondrosarcoma, osteogenic sarcoma, chordoma, angiosarcoma, Kaposi's sarcoma, endotheliosarcoma, lymphangiosarcoma, lymphangioendotheliosarcoma, synovioma, mesothelioma, Ewing's tumor, leiomyosarcoma, rhabdomyosarcoma, rhabdosarcoma, colorectal carcinoma, pancreatic cancer, breast cancer, ovarian cancer, prostate cancer, squamous cell carcinoma, basal cell carcinoma, adenocarcinoma, sweat gland carcinoma, sebaceous gland carcinoma, papillary carcinoma, papillary adenocarcinomas, cystadenocarcinoma, medullary carcinoma, bronchogenic carcinoma, renal cell carcinoma, hepatoma, bile duct carcinoma, choriocarcinoma, seminoma, embryonal carcinoma, Wilms' tumor, cervical cancer, testicular tumor, lung carcinoma, small cell lung carcinoma, bladder carcinoma, epithelial carcinoma, glioma, astrocytoma, medulloblastoma, craniopharyngioma, ependymoma, pinealoma, hemangioblastoma, acoustic neuroma, oligodendroglioma, meningioma, neuroblastoma, retinoblastoma, myeloma, lymphoma, and leukemia cells.
37. The vaccine of claim 35, wherein the cancer cell lines are selected from the group of cancer cell lines consisting of fibrosarcoma, myxosarcoma, liposarcoma, chondrosarcoma, osteogenic sarcoma, chordoma, angiosarcoma, Kaposi's sarcoma, endotheliosarcoma, lymphangiosarcoma, lymphangioendotheliosarcoma, synovioma, mesothelioma, Ewing's tumor, leiomyosarcoma, rhabdomyosarcoma, rhabdosarcoma, colorectal carcinoma, pancreatic cancer, breast cancer, ovarian cancer, prostate cancer, squamous cell

carcinoma, basal cell carcinoma, adenocarcinoma, sweat gland carcinoma, sebaceous gland carcinoma, papillary carcinoma, papillary adenocarcinomas, cystadenocarcinoma, medullary carcinoma, bronchogenic carcinoma, renal cell carcinoma, hepatoma, bile duct carcinoma, choriocarcinoma, seminoma, embryonal carcinoma, Wilms' tumor, cervical cancer, testicular tumor, lung carcinoma, small cell lung carcinoma, bladder carcinoma, epithelial carcinoma, glioma, astrocytoma, medulloblastoma, craniopharyngioma, ependymoma, pinealoma, hemangioblastoma, acoustic neuroma, oligodendroglioma, meningioma, neuroblastoma, retinoblastoma, myeloma, lymphoma, and leukemia cell lines.

38. The vaccine of claim 18, wherein the cancer cells are harvested from a host to be treated with the composition.
39. A method for preparing an immunotherapeutic vaccine, which comprises:
- (a) contacting cancer cells with a recombinant vaccinia virus encoding an immunostimulating molecule;
 - (b) disrupting the vaccinia virus-contacted cancer cells to obtain a preparation comprising enucleated cytosol, and cell membranes from said cancer cells; and
 - (c) pulsing antigen presenting cells with the preparation.
40. The method of claim 39, wherein the contacting step is stopped before the cancer cells are lysed by the vaccinia virus.
41. The method of claim 39, wherein the contacting step lasts between about four hours and about thirty-six hours.
42. The method of claim 39, wherein the contacting step lasts about twenty-four hours.
43. The method of claim 39, wherein the preparation is substantially free of cancer cell nuclei.
44. The method of claim 39, wherein the disrupting step is carried out by physical or chemical means.
45. The method of claim 39, wherein the disrupting step is carried out by sonication.
46. The method of claim 39, wherein the antigen presenting cells are obtained from peripheral blood, spleen, skin, thymus, lymph nodes or bone marrow.
47. The method of claim 46, wherein the antigen presenting cells are HLA-matched to a host to be treated.
48. The method of claim 39, wherein the pulsing step is carried out for a time sufficient to allow the antigens in the preparation to be processed by and presented on the plasma membrane

of the antigen presenting cells.

49. The method of claim 39, wherein the pulsing step is carried out for at least six hours.
50. The method of claim 39, wherein the ratio between the cancer cells and plaque forming units (PFU) of the recombinant vaccinia virus is from about 1 million:1 to about 1:1.
51. The method of claim 39, wherein the ratio between the cancer cells and PFU of the recombinant vaccinia virus is about 10 to 1.
52. The method of claim 39, wherein the ratio between the cancer cells and the antigen presenting cells is about 1,000:1 to about 1:10.
53. The method of claim 39, wherein the ratio between the cancer cells and the antigen presenting cells is from about 10:1 to about 2:1.
54. A method for eliciting an anti-cancer immune response, which comprises administering a therapeutically-effective amount of a composition comprising antigen presenting cells pulsed with a preparation comprising enucleated cytosol and cell membranes of cancer cells infected with a recombinant vaccinia virus encoding at least one immunostimulating molecule.
55. A method for eliciting an anti-cancer immune response in a subject, which comprises:
- (a) administering a first recombinant vaccinia virus encoding at least one first immunostimulating molecule; and
 - (b) administering a composition comprising antigen presenting cells pulsed with a preparation comprising enucleated cytosol and cell membranes of cancer cells infected with a second recombinant vaccinia virus encoding at least one second immunostimulating molecule.
56. The method of claim 56, wherein the amount of the first recombinant vaccinia virus is from about 10^4 to about 10^8 PFU.
57. The method of claim 56, wherein the amount of the first recombinant vaccinia virus is about 10^7 PFU.
58. The method of claim 56, wherein the number of antigen presenting cells is from about 10^5 to about 10^7 .
59. The method of claim 56, wherein the number of antigen presenting cells is from about 10^6 to about 5×10^6 cells.
60. The method of claim 56, wherein the enucleated cytosol is substantially free of nuclei.

61. The method of claim 56, wherein the cell membranes comprise at least two HLA class I A antigens.
62. The method of claim 56, wherein the first recombinant vaccinia virus is a live virus.
63. The method of claim 56, wherein the second recombinant vaccinia virus is either live or inactivated.
64. The method of claim 56, wherein the first immunostimulating molecule is IL-2.
65. The method of claim 56, wherein the first immunostimulating molecule is selected from the group consisting of FLT-3 ligand, FLT-3/FLK-2 ligand, GM-CSF, G-CSF, IL-3, IL-4, IL-6, IL-7, IL-12, IL-15, IL-18, stem cell factor, an interferon, and a combination thereof.
66. The method of claim 18, wherein the first immunostimulating molecule comprises a melanoma immunogen selected from the group consisting of MAGE-1, MAGE-3, BAGE, GAGE, PRAME, NY-ESO-1, tyrosinase, Melan-A, MART- 1, gp 100, TRP- 1, TRP-2, MUM- 1, CDK4, beta-catenin, gp 100in4, p15, N-acetylglucosaminyltransferase, B7-1, TA-90, lysosome-associated membrane protein, melanocyte-stimulating hormone receptor, p90 calnexin, and a combination thereof.
67. The method of claim 56, wherein the second immunostimulating molecule is IL-2.
68. The method of claim 56, wherein the second immunostimulating molecule is selected from the group consisting of FLT-3 ligand, FLT-3/FLK-2 ligand, GM-CSF, G-CSF, IL-3, IL-4, IL-6, IL-7, IL-12, IL-15, IL-18, stem cell factor, an interferon, and a combination thereof.
69. The method of claim 56, wherein the second immunostimulating molecule comprises a melanoma immunogen selected from the group consisting of MAGE-1, MAGE-3, BAGE, GAGE, PRAME, NY-ESO-1, tyrosinase, Melan-A, MART- 1, gp 100, TRP- 1, TRP-2, MUM- 1, CDK4, beta-catenin, gp 100in4, p15, N-acetylglucosaminyltransferase, B7-1, TA-90, lysosome-associated membrane protein, melanocyte-stimulating hormone receptor, p90 calnexin, and a combination thereof.
70. The method of claim 56, wherein the antigen presenting cells are dendritic cells or monocytes.
71. The method of claim 56, wherein the antigen presenting cells are dendritic cells and monocytes.
72. The method of claim 56, wherein the antigen presenting cells are autologous cells.

73. The method of claim 56, wherein the antigen presenting cells are HLA-matched cells to the subject.
74. The method of claim 56, wherein the cancer cells are melanoma cells.
75. The method of claim 75, wherein the melanoma cells comprise one or more cells selected from the group consisting of Mel-2, Mel-3, Mel-4, Mel-6, and Mel-9.
76. The method of claim 56, wherein the cancer cells are established cancer cell lines.
77. The method of claim 56, wherein the cancer cells are from the subject.
78. A method of treating cancer in a subject, which comprises:
- (a) administering a first live recombinant vaccinia virus encoding at least one first immunostimulating molecule; and
 - (b) administering an effective amount of a composition comprising antigen presenting cells pulsed with a preparation comprising enucleated cytosol and cell membranes of cancer cells infected with a second recombinant vaccinia virus encoding for at least one second immunostimulating molecule.
79. The method of claim 78, wherein the first live recombinant vaccinia virus encodes IL-2.
80. The method of claim 78, wherein about 10^5 to about 10^7 PFU of the first live recombinant vaccinia virus is provided.
81. The method of claim 78, wherein enucleated cytosol and cell membranes equivalent to about 10^6 to about 10^7 cancer cells are provided.
82. The method of claim 78, wherein at least one treatment is administered.
83. The method of claim 78, wherein parts said first recombinant vaccinia virus and said composition are injected subcutaneously in at least one site selected from the group consisting of an anterior thigh, an upper arm, or the anterior thorax.
84. The method of claim 78, wherein the at least one site is near regional lymph nodes.
85. The method of claim 78, wherein step (a) is carried out before step (b).
86. The method of claim 85, wherein steps (a) and (b) are carried out in substantially the same site.
87. The method of claim 78, wherein step (a) is carried out about thirty minutes prior to step (b).
88. The method of claim 78, wherein the cancer is a melanoma.
89. The method of claim 78, wherein the cancer cells are melanoma cells.

90. The method of claim 78, wherein the cancer is selected from the group consisting of fibrosarcoma, myxosarcoma, liposarcoma, chondrosarcoma, osteogenic sarcoma, chordoma, angiosarcoma, Kaposi's sarcoma, endotheliosarcoma, lymphangiosarcoma, lymphangioendotheliosarcoma, synovioma, mesothelioma, Ewing's tumor, leiomyosarcoma, rhabdomyosarcoma, rhabdosarcoma, colorectal carcinoma, pancreatic cancer, breast cancer, ovarian cancer, prostate cancer, squamous cell carcinoma, basal cell carcinoma, adenocarcinoma, sweat gland carcinoma, sebaceous gland carcinoma, papillary carcinoma, papillary adenocarcinomas, cystadenocarcinoma, medullary carcinoma, bronchogenic carcinoma, renal cell carcinoma, hepatoma, bile duct carcinoma, choriocarcinoma, seminoma, embryonal carcinoma, Wilms' tumor, cervical cancer, testicular tumor, lung carcinoma, small cell lung carcinoma, bladder carcinoma, epithelial carcinoma, glioma, astrocytoma, medulloblastoma, craniopharyngioma, ependymoma, pinealoma, hemangioblastoma, acoustic neuroma, oligodendroglioma, meningioma, neuroblastoma, retinoblastoma, myeloma, lymphoma, and leukemia.
91. The method of claim 78, wherein the cancer cells are from cancers selected from the group consisting of fibrosarcoma, myxosarcoma, liposarcoma, chondrosarcoma, osteogenic sarcoma, chordoma, angiosarcoma, Kaposi's sarcoma, endotheliosarcoma, lymphangiosarcoma, lymphangioendotheliosarcoma, synovioma, mesothelioma, Ewing's tumor, leiomyosarcoma, rhabdomyosarcoma, rhabdosarcoma, colorectal carcinoma, pancreatic cancer, breast cancer, ovarian cancer, prostate cancer, squamous cell carcinoma, basal cell carcinoma, adenocarcinoma, sweat gland carcinoma, sebaceous gland carcinoma, papillary carcinoma, papillary adenocarcinomas, cystadenocarcinoma, medullary carcinoma, bronchogenic carcinoma, renal cell carcinoma, hepatoma, bile duct carcinoma, choriocarcinoma, seminoma, embryonal carcinoma, Wilms' tumor, cervical cancer, testicular tumor, lung carcinoma, small cell lung carcinoma, bladder carcinoma, epithelial carcinoma, glioma, astrocytoma, medulloblastoma, craniopharyngioma, ependymoma, pinealoma, hemangioblastoma, acoustic neuroma, oligodendroglioma, meningioma, neuroblastoma, retinoblastoma, myeloma, lymphoma, and leukemia.
92. The method of claim 78, wherein the enucleated cytosol is substantially free of nuclei.
93. The method of claim 78, wherein the cell membranes comprise at least two HLA class I A antigens.

94. The method of claim 78, wherein the first recombinant vaccinia virus is either live or inactivated.
95. The method of claim 78, wherein the second recombinant vaccinia virus is either live or inactivated.
96. The method of claim 78, wherein the first immunostimulating molecule is IL-2.
97. The method of claim 78, wherein the first immunostimulating molecule is selected from the group consisting of FLT-3 ligand, FLT-3/FLK-2 ligand, GM-CSF, G-CSF, IL-3, IL-4, IL-6, IL-7, IL-12, IL-15, IL-18, stem cell factor, an interferon, and a combination thereof.
98. The method of claim 78, wherein the first immunostimulating molecule comprises a melanoma immunogen selected from the group consisting of MAGE-1, MAGE-3, BAGE, GAGE, PRAME, NY-ESO-1, tyrosinase, Melan-A, MART- 1, gp 100, TRP- 1, TRP-2, MUM- 1, CDK4, beta-catenin, gp 100in4, p15, N-acetylglucosaminyltransferase, B7-1, TA-90, lysosome-associated membrane protein, melanocyte-stimulating hormone receptor, p90 calnexin, and a combination thereof.
99. The method of claim 78, wherein the second immunostimulating molecule is IL-2.
100. The method of claim 78, wherein the second immunostimulating molecule is selected from the group consisting of FLT-3 ligand, FLT-3/FLK-2 ligand, GM-CSF, G-CSF, IL-3, IL-4, IL-6, IL-7, IL-12, IL-15, IL-18, stem cell factor, an interferon, or a combination thereof.
101. The method of claim 78, wherein the second immunostimulating molecule comprises a melanoma immunogen selected from the group consisting of MAGE-1, MAGE-3, BAGE, GAGE, PRAME, NY-ESO-1, tyrosinase, Melan-A, MART- 1, gp 100, TRP- 1, TRP-2, MUM- 1, CDK4, beta-catenin, gp 100in4, p15, N-acetylglucosaminyltransferase, B7-1, TA-90, lysosome-associated membrane protein, melanocyte-stimulating hormone receptor, p90 calnexin, and a combination thereof.
102. The method of claim 78, wherein the antigen presenting cells are dendritic cells or monocytes.
103. The method of claim 78, wherein the antigen presenting cells are dendritic cells and monocytes.
104. The method of claim 78, wherein the antigen presenting cells are autologous cells.
105. The method of claim 78, wherein the antigen presenting cells are HLA-matched to the

subject.

106. The method of claim 78, wherein the cancer cells are from the subject.

107. The method of claim 78, wherein the subject is a human.

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